

### **Remarks**

Applicants respond to the Final Office Action dated February 26, 2009, for which a three month period of response is given. Upon entry of the foregoing amendment, claims 1, 4-5, 7-8, 16-19 and 34 will be pending in the application. Claims 2, 3, 6, 9-15, 20-33 and 35-40 have been canceled. Claims 1, 4 and 34 have been amended.

As suggested by the Examiner, claim 4 has been amended to replace "an amino acid sequence" with "the amino acid sequence".

### **Claim Rejections – 35 USC §112**

Claims 1, 3-5, 7-8, 16-19 and 34 have been rejected under 35 U.S.C. §112, first paragraph. The Examiner contends that the specification does not provide enablement for the natural  $\alpha$ -glucan phosphorylase (SEQ ID NO: 2) having one or several amino acids that are deleted, substituted or added relative to an amino acid sequence of natural  $\alpha$ -glucan phosphorylase, wherein the enzyme activity of the  $\alpha$ -glucan phosphorylase has improved thermostability that is equivalent or superior to the natural  $\alpha$ -glucan phosphorylase (claims 1 and 34); or wherein the natural  $\alpha$ -glucan phosphorylase has varying sequence homology of at least 50% identity with position 1 to position 916 of SEQ ID NO: 2 (claim 3); or wherein the amino acid sequence of the natural  $\alpha$ -glucan phosphorylase of SEQ ID No: 2 hybridizes under stringent conditions (claim 4).

Claims 1, 3-5, 7-8, 16-19 and 34 have also been rejected under 35 U.S.C. §112, first paragraph as failing to comply with the written description requirement. The Examiner contends that there is no teaching in the specification regarding the 50% or more of the structure of SEQ ID NO: 2 that can be varied while retaining the ability of the protein to function as an  $\alpha$ -glucan phosphorylase enzyme and be thermostable.

Applicants respectfully traverse the rejections. Claims 1 and 34 have been amended to clarify that the  $\alpha$ -glucan phosphorylase is a modified  $\alpha$ -glucan phosphorylase. This amendment is supported by the specification on page 70, line 28, and page 123, line 19 to page 127, line 9. Claims 1 and 34 have been amended to recite that the  $\alpha$ -glucan phosphorylase has an amino acid substitution at a position corresponding to position 7 in a motif sequence 3L: R-I-V-K-F-I-T-D-V (SEQ ID NO: 47)

and said substitution is a substitution to C, I, L, V or W. This amendment is supported by the specification on page 12, lines 16-20 and page 123, line 19 to page 142, line 13.

Claims 1 and 34 have been further amended to recite that the natural  $\alpha$ -glucan phosphorylase has an amino acid sequence which is at least 50% identical to the sequence of SEQ ID NO: 2 (residues 1-916). This amendment is supported by the specification on page 9, lines 2-5. In Claims 1 and 34, it has been recited that the  $\alpha$ -glucan phosphorylase having improved thermostability has an amino acid sequence which is at least 95% identical to the sequence of the natural  $\alpha$ -glucan phosphorylase. This amendment is supported by the specification on page 86, lines 4-14.

The modified  $\alpha$ -glucan phosphorylase of the present invention is obtained by modifying a natural  $\alpha$ -glucan phosphorylase which has an amino acid sequence that is at least 50% identical to the sequence of SEQ ID NO: 2 (residues 1-916). The natural  $\alpha$ -glucan phosphorylase may be any naturally-occurring  $\alpha$ -glucan phosphorylase having  $\alpha$ -glucan phosphorylase activity. The natural  $\alpha$ -glucan phosphorylase would not be obtained by modifying the amino acid sequence of SEQ ID NO: 2 (residues 1-916). One skilled in the art would be able to obtain an active natural enzyme which has an amino acid sequence at least 50% identical to a specified amino acid sequence of an enzyme from a natural source. The natural  $\alpha$ -glucan phosphorylase to be modified is active. Thus, non-active proteins having an amino acid sequence which is at least 50% identical to the sequence of SEQ ID NO: 2 (residues 1-916) have been excluded from the scope of the present invention.

The phrase "wherein the natural  $\alpha$ -glucan phosphorylase has an amino acid sequence which is at least 50% identical to the sequence of SEQ ID NO: 2 (residues 1-916)" does not intend to include any artificially modified  $\alpha$ -glucan phosphorylase having at least 50% identical to the sequence of SEQ ID NO: 2 (residues 1-916) in the scope of the natural  $\alpha$ -glucan phosphorylase.

Although the modified  $\alpha$ -glucan phosphorylase of the present invention can have minor modifications other than the substitution at the specified position, the essential modification is the substitution at a position corresponding to position 7 in a motif sequence 3L: R-I-V-K-F-I-T-D-V (SEQ ID NO: 47). It was found by the present

inventors that the substitution at this position to the specified residues affects and improves thermostability of the natural  $\alpha$ -glucan phosphorylase. One amino acid substitution at this position can affect and improve thermostability of the natural  $\alpha$ -glucan phosphorylase as described in the present specification. Making this substitution would not involve undue experimentation for those skilled in the art who have read the present specification.

As apparent from the phrase "wherein the  $\alpha$ -glucan phosphorylase having improved thermostability has an amino acid sequence which is at least 95% identical to the sequence of the natural  $\alpha$ -glucan phosphorylase," the modified  $\alpha$ -glucan phosphorylase of the present invention can have only minor modifications with respect to the natural  $\alpha$ -glucan phosphorylase. As discussed in the previous response dated November 17, 2008, the amino acid residues essential for glucan phosphorylase activity in the amino acid sequence of glucan phosphorylase was known in the art at the time of filing the present application (for example, see Exhibit 2 which was filed in the previous response). Based on this knowledge of the essential residues, such minor modifications would be routinely made or identified by those skilled in the art. Thus, the present specification fulfills the enablement requirement and written description requirement. Accordingly, Applicants respectfully request withdrawal of the rejections under 35 U.S.C. §112, first paragraph.

#### **Claim Rejection – 35 USC §102**

Claims 1, 3-5, 7-8, 16-19 and 34 have been rejected under 35 U.S.C. §102(b) as being anticipated by Accession No. Q9LKJ3 (2000), which is an  $\alpha$ -glucan phosphorylase that is at least 50% identical to the sequence of SEQ ID NO: 2, and wherein position 7 in motif "RIVKFIIDV" is "N". The Examiner contends that the  $\alpha$ -glucan phosphorylase of Accession No. Q9LKJ3 inherently possesses improved thermostability and the other limitations recited in the claims.

Applicants respectfully traverse the rejection. As recited in Claims 1 and 34, the  $\alpha$ -glucan phosphorylase having improved thermostability has an amino acid substitution at a position corresponding to position 7 in a motif sequence 3L: R-I-V-K-F-I-T-D-V (SEQ ID NO: 47); wherein said substitution is a substitution to C, I, L, V or W. On the

other hand,  $\alpha$ -glucan phosphorylase of Accession No. Q9LKJ3 (2000) has "N" in the position corresponding to position 7 in a motif sequence 3L: R-I-V-K-F-I-T-D-V (SEQ ID NO: 47). Thus, the modified  $\alpha$ -glucan phosphorylase of the present invention is different from the  $\alpha$ -glucan phosphorylase of Accession No. Q9LKJ3 (2000). Accession No. Q9LKJ3(2000) does not teach nor suggest the substitution at a position corresponding to position 7 in a motif sequence 3L into a substitution to C, I, L, V or W. Accordingly, claims 1, 4-5, 7-8, 16-19 and 34 are not anticipated by or rendered obvious by Accession No. Q9LKJ3 (2000). Applicants respectfully request withdrawal of the rejection under 35 U.S.C. §102(b).

### **Conclusion**

In view of the foregoing amendments and remarks, it is believed that the application is in condition for allowance and a notice of allowance is therefore respectfully requested.

In the event any fee or additional fee is due in connection with the filing of this paper, the Commissioner is authorized to charge those fees to our Deposit Account No. 18-0988 (under Docket Number YAMAP0997US). In the event an extension of time is needed to make the filing of this paper timely and no separate petition is attached, please consider this a petition for the requisite extension and charge the fee to our Deposit Account No. 18-0988 (under Docket Number YAMAP0997US).

Respectfully submitted,

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